PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

07 SEP 2004

Applicant's or agent's file reference CG/RM/02157	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/IB 03/01293	International filing date *(day/month/year)* 07.03.2003	Priority date *(day/month/year)* 07.03.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/76		
Applicant SVANBORG, Catharina et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.10.2003	07.06.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Helduschat, C Telephone No. +49 89 2399-7804

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB 03/01293

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-39 as originally filed

Claims, Numbers

1-14 as originally filed

Drawings, Sheets

1/13-13/13 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5,6,10
	No:	Claims	1-4, 7-9, 11-14
Inventive step (IS)	Yes:	Claims	none
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	13-14

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/IB03/01293

Re Item I

Basis of the Report

The amendments filed with the fax dated 14.04.2004 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: Claims 1 and 5 comprise an extended proviso not only excluding a complex comprising native alpha-lactalbumin and a C18:1:9 cis fatty acid but also complexes of said fatty acid with a full-length alpha-lactalbumin or a variant therof comprising a modification of the calcium binding site. No basis was found for this extended proviso in the International PCT Application as originally filed.
Therefore this International Preliminary Examination Report is established on the basis of the originally filed claims.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) The Application
The present application is based on a complex comprising alpha-lactalbumin in the apo folding state and a certain fatty acid as stabilizing cofactor.

2) The Prior Art
Reference is made to the following document/s/:

D1: WO 99 26979 A (HAAKANSSON PER ANDERS ;SVENSSON MALIN WILHELMINA (SE); SVANBORG CA) 3 June 1999

D2: M. SVENSSON ET AL: 'Conversion of alpha-lactalbumin to a protein inducing apoptosis' PNAS, vol. 97, no. 8, 11 April 2000 , pages 4221-4226, XP002250705

D3: ANDERS HAKANSSON ET AL: 'A folding variant of alpha-lactalbumin with bactericidal activity against Streptococcus pneumoniae' MOLECULAR MICROBIOLOGY, vol. 35, no. 3, 2000, pages 589-600, XP002250706

D4: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; October 2001 PERMYAKOV SERGEI E ET AL: 'Mutating aspartate in the calcium-binding site of alpha-lactalbumin: Effects on the protein stability and cation binding.' XP002250707 & PROTEIN ENGINEERING, vol. 14, no. 10, October 2001 (2001-10), pages 785-789,

3) Novelty

The subject-matter of claims 1 to 4, 7 to 9 and 11 to 14 cannot be considered novel in the sense of Article 33(2) PCT for the following reasons.

D1 describes a method to produce complexes of alpha-lactalbumin in the molten globule-like state which also includes the addition of oleic acid (C18:1:9 cis) (p.5, l.32-p.7, l.35). Such complexes were already described to have applications as antibiotics and in anti-cancer therapy (p.1, l.21-34). D1 also suggests variants of alpha-lactalbumin comprising mutations destroying the calcium binding site and gives a specific example (see p.28 to 29, Example 11).

D2 and D3 describe a folding variant of alpha-lactalbumin (apo-alpha-lactalbumin) which is stabilised by a C18:1 fatty acid. This variant can be used to induce apoptosis in tumorigenic cells (see D2) and shows bactericidal activity against Streptococcus pneumoniae (see D3).

Thus, each of D1, D2 and D3 is considered novelty destroying to claims 1 to 4, 7 to 9, 11 and 12. In addition D1 and D2 are each considered novelty destroying to claim 13 and each of D1 and D3 is considered novelty destroying to claim 14.

4) Inventive Step

4.1 Document D1 which is considered to represent the most relevant state of the art, discloses complexes of alpha-lactalbumin in the molten-globule like state that are derived from native alpha-lactalbumin or from variants mutated at cystein residues 61, 73, 77 and 91.

4.2 The problem to be solved by the present invention may therefore be regarded as the provision of alternative variants of alpha-lactalbumin favouring the change in conformation.

4.3 The solution provided by the present application consists in mutations at residues D82, D87, D88, K79 or D84 within the Ca^{2+} binding site of alpha-lactalbumin, which allow for abolished or reduced Ca^{2+} binding affinity and for retention in the apo conformation. Thus the problem is considered solved.

4.4 D1 already suggested that mutations abolishing Ca^{2+} binding may favour the molten-globule formation of alpha-lactalbumin. It even refers to specific residues D82, D87, D88, K79 and D84 known from the literature (p.1 and 3). These residues are also suggested by D2.

4.5 D4 describes the effect of mutations D87A and D87N on Ca^{2+} binding affinity. Thus, said specific substitutions were suggested by the prior art.

4.6 The mutation of R70 to S70 of bovine alpha-lactalbumin (see claim 10) outside of the

Ca^{2+} binding domain was not suggested by any of documents D1 to D4. However, it is not apparent, which problem is actually solved by this mutation.

4.7 Neither the solution proposed in claims 5 or 6 nor in claim 10 of the present application can be considered as involving an inventive step (Article 33(3) PCT).

5) Industrial Applicability

Claims 13 to 14 may be understood as methods of medical treatment. For the assessment of said claims on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims

1. A biologically active complex comprising alpha-lactalbumin or a variant of alpha-lactalbumin (α-lactalbumin) which is in the apo folding state, or a fragment of either of any of these, and a cofactor which stabilises the complex in a biologically active form, provided that any fragment of α-lactalbumin or a variant thereof comprises a region corresponding to the region of α-lactalbumin which forms the interface between the alpha and beta domains, and further provided that when the complex comprises full length α-lactalbumin or a variant of α-lactalbumin in which the calcium binding site has been modified so that the affinity for calcium is reduced, or it is no longer functional, the cofactor is other than C18:1:9 cis fatty acid.

2. A complex according to claim 1 wherein the cofactor is a cis C18:1:9 or C18:1:11 fatty acid or a different fatty acid with a similar configuration.

3. A complex according to claim 1 or claim 2 wherein the cofactor is C18:1:11 fatty acid.

4. A complex according to any one of claims 1 to 3 which comprises a fragment of α-lactalbumin or a variant thereof, which fragment includes a region corresponding to the region of α-lactalbumin which forms the interface between the alpha and beta domains.

5. A biologically active complex according to claim 1 which is obtainable by combining
(i) a cis C18:1:9 or C18:1:11 fatty acid or a different fatty acid with a similar configuration; and
(ii) α-lactalbumin from which calcium ions have been removed, or a variant of α-lactalbumin from which calcium ions have been

removed or which does not have a functional calcium binding site; or a fragment of either of any of these, provided that any fragment comprises a region corresponding to the region of α-lactalbumin which forms the interface between the alpha and beta domains, and further provided that when (ii) is full length α-lactalbumin or a variant of α-lactalbumin in which the calcium binding site has been modified so that the affinity for calcium is reduced, or it is no longer functional, (i) is other than C18:1:9 cis fatty acid.

6. A complex according any one of claims 1 to 5 which includes a variant of α-lactalbumin in which the calcium binding site has been modified so that the affinity for calcium is reduced, or it is no longer functional, and in which the cofactor is C18:1:11 fatty acid..

7. A complex according to claim 6 wherein the variant has a mutation at a position corresponding to at least one of the K79, D82, D84, D87 or D88 residues.

8. A complex according to claim 7 which includes a D87A or D87N variant of α-lactalbumin .

9. A complex according to any one of the preceding claims which comprises a fragment of α-lactalbumin or a variant thereof, and where the fragment includes the entire region from amino acid 34-86 of the native protein.

10. A complex according to any one of the preceding claims wherein the α-lactalbumin is human or bovine α-lactalbumin or a variant of either of these.

11. A complex according to claim 10 wherein the α-lactalbumin is human α-lactalbumin.

12. A complex according to claim 11 wherein the α-lactalbumin is mutant bovine α-lactalbumin which includes an S70R mutation.

13. A complex according to any one of the preceding claims which further comprises calcium ions.

14. A pharmaceutical composition comprising a complex according to any one of the preceding claims in combination with a pharmaceutically acceptable carrier.

15. A method for treating cancer which comprises administering to cancer cells a complex according to any one of claims 1 to 13 or a composition according to claim 14.

16. A method for treating bacterial infections which comprises administering to a patient in need thereof, a complex or a composition as described above.